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"Studies on PGPR for Growth Promotion of Gidhya Sag A Medicinal Species in Painganga Wildlife Sanctuary"

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Studies on PGPR for Growth Promotion of Gidhya Sag A Medicinal Species in Painganga Wildlife Sanctuary

1.Introduction:

Gidhya Sag *Hymenodictyon obovatum* Wall a member of family Rubiaceae, is a medicinally important plant, located in Painganga Wildlife Sanctuary, Yavatmal District, Tahasil Umerkhed covering up to 325 sq/km area. It is observed that in recent time, the plant has become very rare. *Hymenodictyon obovatum* Wall (Gidhya sag) is very useful and important medicinal plant used largely by local tribes and medicinal practitioners. Currently due to over use by local tribes and medicinal practitioners, some diseased condition and unfavourable environmental condition that might have become endangered. So it needs to be conserved and multiplied. Efforts should be taken to enhance the quantity of *Hymenodictyon obovatum* Wall in Painganga wildlife sanctuary.

During survey of Painganga wild life sanctuary, the medicinal plant locally named as Gidhya sag (*Hymenodictyon obovatum* Wall*),* found in Painganga wildlife sanctuary. Gidhya sag is (*Hymenodictyon obovatum* Wall*)* used as antidiabetic medicine, the bark of the tree is used as water purifier, also for fever, dysentery and vomiting. Discussion with local tribes and other medicinal practitioners and forest officials concludes that the population of the plants of Gidhya sag has decreased in recent times, due to excess use of medicinal parts, unproper training of medicine collection, forest fires, climate changes, very low germination rate and some diseases. Local tribes use this plant for many years, but during last 10 to 15 years the population of the plant has continuously decreased in number.

Because of these facts, present study mainly focuses on growth enhancement and germination of seeds of *Hymenodictyon obovatum* Wall by using plant Growth Promoting Rhizospheric Bacteria (PGPRB).

2. Review of Literature and Development in the subject:

The present work is planned for searching of Gidhya sag growth promoting rhizospheric bacteria, particularly Phosphate Solubilizing Bacteria (PSB), Potassium Solubilizing Bacteria (KSB) and Nitrogen Fixing Bacteria (NFB) which also will be screened for their efficacy as a biocontrol against Gidhya sag disease.

Several microorganisms belonging to the rhizospheric niches of plants includes Plant Growth-Promoting Rhizobacteria (PGPR's including -Nitrogen Fixing Bacteria, Phosphate Solubilizing Bacteria and Potassium Solubilizing bacteria) and Cyanobacteria are known to be essential symbionts without which the vast majority of plant could not survive in soils with normal levels of available phosphorus and nitrogen. Additional benefits from this microbiological flora as to develop resistance against major diseases in several crops.

Due to negative environmental impacts of chemical fertilizers and increasing costs, the utilization of plant growth promoting rhizospheric bacteria (PGPRB) is advantageous for sustainable agricultural practices (Gyaneshwar *et al.*, 2002).

i) Phosphate Solubilizing Bacteria (PSB):

Vast amount of Phosphate Solubilizing Bacteria available naturally in the rhizospheric niches of plants. Phosphate solubilizing bacteria play an important role in supplementing phosphorus to plants, allowing sustainable use of phosphate (Bhattacharya *et al.,* 2000). Soil and seed inoculation with phosphate solubilizing bacteria (PSB) improves solubilization of fixed soil phosphorus and of applied phosphates, resulting in higher crop yields (Jones and Darrah, 1994; Toro *et al.,* 1997).

In 2014, Nadeem *et al*., found that Plant growth under stress conditions may be enhanced by the application of microbial inoculation including plant growth promoting rhizobacteria (PGPR) and mycorrhizal fungi and can promote plant growth by regulating nutritional and hormonal balance, producing plant growth regulators, solubilizing nutrients and inducing resistance against plant pathogens. However, literature survey revealed that research is still required to obtain the practical effectiveness of microbiological sources for growth promotion and biocontrol.

ii) Nitrogen Fixing Bacteria (NFB):

Similarly, several authors reported that Nitrogen Fixing Bacteria (NFB) can be used for growth promotion of crop plants. Some of them are:

Yu *et al.,* (2012) evaluated the effects of co-inoculation of Phosphate-Solubilizing Bacteria (PSB) and Nitrogen-Fixing Bacteria (NFB) on growth promotion of walnut seedlings. They tested two PSB strains, *Pseudomonas chlororaphis* and *Bacillus megaterium*, and two NFB strains, *Arthrobacter pascens* and *Burkholderia cepacia,* Co-inoculation with *P. chlororaphis* and *A. pascens* amendment with RP resulted in the highest plant height, shoot and root dry weight, P and nitrogen (N) uptake of walnut seedlings, and the maximum amounts of available P and N in soils under shade house conditions. However, mixed inoculation with *B. megaterium* and *A. pascens* failed to increase these parameters.

Islam *et al.,* (2013) reported that 13 nitrogen-fixing bacterial strains belonging to 11 different genera were tested for effective plant growth promotion in tomato and red pepper. All of the strains were positive for 1-aminocyclopropane-1-carboxylate deaminase (ACCD), indole-3-acetic acid (IAA), salicylic acid and ammonia production while negative for cellulase, pectinase, and hydrocyanic acid production. The *Pseudomonas* spp. RFNB3 and *Serratia* spp. RFNB14 strains were the most effective in solubilizing both tri-calcium phosphate and zinc oxide. In addition, all strains except *Pseudomonas* sp. RFNB3 were able to oxidize sulfur, and six strains were positive for siderophore synthesis. Each strain tested in this study possesses at least four PGP properties in addition to nitrogen fixation. Nine strains were selected based on their multiple PGP potential, particularly ACCD and IAA production, and evaluated for their effects on early growth of tomato and red pepper under gnotobiotic conditions. Bacterial inoculation considerably influenced root and shoot length, seedling vigor, and dry biomass of the two crop plants. Three strains that demonstrated substantial effects on plant performance were further selected for greenhouse trials with red pepper, and among them *Pseudomonas* sp. RFNB3 resulted in significantly higher plant height (26%) and dry biomass (28%) compared to control. The highest rate of nitrogen fixation, as determined by acetylene reduction assay, occurred in *Novosphingobium* sp. RFNB21 inoculated red pepper root (49.6 nM of ethylene/h/g of dry root) and rhizosphere soil (41.3 nM of ethylene/h/g of dry soil). Inoculation

with nitrogen-fixing bacteria significantly increased chlorophyll content, and the uptake of different macro and micro-nutrient contents enhancing also in red pepper shoots, in comparison with uninoculated controls. The population estimation studies showed that nitrogen-fixing as well as total heterotrophic bacteria were also noticeably increased in soil and plant samples. The findings of this study suggest that certain nitrogen-fixing strains possessing multiple PGP traits could be applied in the development of biofertilizers.

iii) Potassium Solubilizing Bacteria (KSB):

After nitrogen (N) and phosphorus (P), potassium (K) is also the most important plant nutrient that has a key role in the growth, metabolism and development of plants. In addition to increasing plant resistance to diseases, pests, and abiotic stresses (Karley and White, 2010; Gundala *et al.,* 2013; Almeida *et al.,* 2015; Yang *et al.,* 2015; Hussain *et al.,* 2016) K solubilization is done by a wide range of saprophytic bacteria, fungal strains and actinomycetes (Meena *et al.,* 2014; Ahmad *et al.,* 2016; Bakhshandeh *et al.,* 2017).

There is strong evidence that soil bacteria are capable of transforming soil insoluble K to the soluble K forms which make available to plant effectively (Meena *et al.,* 2014; Meena *et al.,* 2015; Meena *et al.,* 2016). The ability to solubilize the silicate rocks by *B. mucilaginosus, B. circulanscan, B. edaphicus, Burkholderia, A. ferrooxidans, Arthrobacter* spp., *Enterobacter hormaechei, Paenibacillus mucilaginosus, P. frequentans, Cladosporium, Aminobacter, Sphingomonas, Burkholderia, and Paenibacillus glucanolyticus* has been reported (Meena *et al.,* 2016). Among the soil bacterial communities, *B. mucilaginosus, B. edaphicus* and *B. circulanscan* have been described as effective K solubilizers (Meena *et al.,* 2014; Meena *et al.,* 2015; Meena *et al.,* 2016).

KSB are usually present in all soils and have been isolated from rhizosphere soil, non-rhizosphere soil, paddy soil (Bakhshandeh *et al.,* 2017) and saline soil (Bhattacharya *et al.,* 2016)

Etesami *et al., (*2017) reported that many bacteria such as *Acidithiobacillus ferrooxidans*, *Paenibacillus* spp., *Bacillus mucilaginosus, B. edaphicus,* and *B. circulans* have capacity to solubilize K minerals (e.g., biotite, feldspar, illite, muscovite, orthoclase, and mica). KSB are usually present in all soils, although their number, diversity and ability for K solubilization vary depending upon the soil and climatic conditions. KSB can dissolve silicate minerals and release K through the production of organic and inorganic acids, acidolysis, polysaccharides, complexolysis, chelation, and exchange reactions. Hence, the production and management of biological fertilizers containing KSB can be an effective alternative to chemical fertilizers.

A wide range of microorganisms found in the rhizosphere are able to produce substances that regulate plant growth and development. Plant growth promoting rhizobacteria produce phytohormones such as Auxins, Cytokinin, Gibberellins and Ethylene can affect cell proliferation in the root architecture by overproduction of lateral roots and root hairs with a subsequent increase of nutrient and water uptake (Arora *et al.,* 2013).

Growth Promoting Parameter of PGPRB:

Plant Growth Promoting Rhizospheric Bacteria (PGPRB) prominently uses following parameters to promote the growth of plants.

Indole Acetic Acid (IAA):

Among plant growth regulators, indole acetic acid (IAA) is the most common natural auxin found in plants and its positive effect on root growth (Arora *et al.,* 2014). Up to 80% of rhizobacteria can synthesize indole acetic acid (IAA) colonized the seed or root surfaces is proposed to act in conjunction with endogenous IAA in plant to stimulate cell proliferation and enhance the host's uptake of minerals and nutrients from the soil (Vessey, 2003). Indole acetic acid affects plant cell division, extension, and differentiation; stimulates seed and tuber germination; increases the rate of xylem and root development; controls processes of vegetative growth; initiates lateral and adventitious root formation; mediates responses to light, gravity and florescence; affects photosynthesis, pigment formation, biosynthesis of various metabolites, and resistance to stressful conditions (Spaepen, 2014).

Tryptophan is an amino acid commonly found in root exudates, has been identified as main precursor molecule for biosynthesis of IAA in bacteria. The biosynthesis of indole acetic acid by plant growth promoting rhizobacteria involves formation via indole-3- pyruvic acid and indole-3-acetic aldehyde, which is the most common mechanism in bacteria like *Pseudomonas, Rhizobium, Bradyrhizobium, Agrobacterium, Enterobacter* and *Klebsiella* (Shilev, 2013). Root growth promotion by the free living PGPR e.g., *Alkaligenes faecalis*, *Enterobacter cloacae*, *Acetobacter dizotrophicous*, species of *Azospirillum*, *Pseudomonas* and Xanthomonas sp. has been related to low level of IAA secretion. However, microbially produced phytohormones are more effective due to the reason that the threshold between inhibitory and stimulatory levels of chemically produced hormones is low, while microbial hormones are more effective by virtue of their continuous slow release.

Cytokinins and Gibberellins:

Several plant growth promoting rhizobacteria *Azotobacter sp., Rhizobium sp., Pantoea agglomerans, Rhodospirillum rubrum, Pseudomonas fluorescens, Bacillus subtilis* and *Paenibacillus polymyxa* can produce cytokinins or gibberellins or both can produce either cytokinins or gibberellins or both for plant growth promotion (Kang *et al.,* 2010). Some strains of phytopathogens can also synthesize cytokinins. However, it appears that plant growth promoting rhizobacteria produce lower cytokinin levels compared to phytopathogens so that the effect of the plant growth promoting rhizobacteria on plant growth is stimulatory while the effect of the cytokinins from pathogens is inhibitory. Ethylene is a key phytohormone has a wide range of biological activities can affect plant growth and development in a large number of different ways including promoting root initiation, inhibiting root elongation, promoting fruit ripening, promoting lower wilting, stimulating seed germination, promoting leaf abscission, activating the synthesis of other plant hormones (Glick *et al.,* 2007). The high concentration of ethylene induces defoliation and other cellular processes that may lead to reduced crop performance (Bhattacharyya *et al.,* 2012).

3. Objectives of the Research:

Considering the importance of Gidhya sag in day to-day life of local tribes nearby Painganga wildlife Sanctuary, following objectives set for growth promotion of Gidhya sag (*Hymenodictyon obovatum* Wall*)* rare species in Painganga wildlife sanctuary by using PGPRB (PSB, NFB and KSB) and also to study in detail the biocontrol abilities of these PGPRB's

against major phytopathogens of Gidhya sag (*Hymenodictyon obovatum* Wall*)* in a cost-effective and eco-friendly manner.

The objectives are as follows:

1. Isolation of PGPRB from rhizospheric niches of healthy plant.
2. Identification of effective PGPRB isolates.
3. Deciphering of Mechanism of PGPRB isolates for Growth Promotion.
4. Pot assay for growth promotion of Gidhya sag by using PGPRB isolates.
5. Isolation of phytopathogens from Diseased parts of Gidhya sag.
6. Screening of PGPRB isolates against phytopathogens for biocontrol ability.
7. Pot assay for biocontrol ability of PGPRB isolates against phytopathogens.

Hypothesis:-

Gidhya sag being medicinally important to local tribe at Painganga wildlife sanctuary, it is necessary to improve the growth of this plant in that aera. So in this research, we are trying to improve the growth of Gidhya sag by using Plant Growth Promoting Rhizospheric Bacteria.

4. Methodology:

In order to investigate the problem following methodology will be adopted:

i) Isolation of PGPRB (PSB and KSB) from rhizospheric niches:

To isolate rhizospheric PGPRB, rhizospheric soil samples from different healthy plants will be collected in polythene bags. Further this rhizospheric soil samples will be used for isolation of PSB by dilution plate technique using Pikovskaya medium (Pikovskaya, 1948) containing tri-calcium phosphate (TCP) (Gupta *et al.,* 2012, Sharma *et al.,* 2014). Also, KSB isolated by serial dilution plate method using modified Aleksandrov medium (Etesami *et al.,* 2017).

ii) Identification of effective PGPRB isolates:

After isolation and purification of the microbial isolates, a detailed general characterization was made using standard microbiological techniques for their relevance and confirmation according to the "Bergey's Manual for systematic Bacteriology" (Boone *et al*., 2005) for bacteria and confirmed by 16s rRNA sequencing method.

iii) Deciphering of mechanism of PGPRB isolates:

To find the Phosphate solubilizing and Potassium solubilizing mechanism the PGPR's isolates tested for organic, inorganic acids production as well as growth promoting parameters such as IAA, Gibberellin and cytokinin production.

iv) Pot assay for growth promotion of tomato by using PGPR's isolates:

Growth promoting ability of selected PGPR's will be analyzed by direct inoculation in pot or seed treatment method and studying different parameters.

v) Isolation of phytopathogens from diseased Gidhya sag:

Isolation of phytopathogens from diseased Gidhya sag plant carried out by following the method of Rashid *et al.,* (2016).

vi) Screening of PGPRB isolates against Gidhya sag phytopathogens for biocontrol ability.

All the PGPR isolates will be screened for antagonistic activity against Phytopathogens of Tomato, using King's B agar by the Dual Culture Technique (Rakh *et al.,* 2011).

vii) Pot assay for biocontrol ability of PGPRB isolates against phytopathogens:

By using the Pot Assay, the microbial control ability of selected PGPRB to confirm the result of plate assay in lab to land according to Yaqub and Shahzad (2005).

5. Importance of study: -

Following this the importance of study:

Conservation of Medicinal Plants: Medicinal plant conservation strategies are based on an understanding of indigenous knowledge and practices. Conservation of medicinal plants in its bio-cultural perspective not only implies conservation of biodiversity but also provides conservation of cultural diversity. As per the world conservation strategy (1980), conservation is defined as "the management of human use of the biodiversity so that it may yield the greatest sustainable benefit to present generation while maintaining its definitions invokes two complementary components conservation and sustainability". Many drugs contain herbal ingredients and it has been said that 80-90% of the world's population relies on some form of non-conventional medicine, but the market demand has led to increased pressure on the natural resources that lend to the production of some of these plants. The most serious proximate threats when extracting medicinal plants generally are habitat loss, habitat degradation, global climate change, and over-harvesting. Hence highly threatened species must not be collected at all to increase their prospects of survival. There should be clear information on which plants are threatened. In such cases, the practitioners and communities should try to discover ways of using alternatives. Additionally, legislation should be put in place to ban the collection and the possession of and trade in those plants. The practitioners should be involved in the processes of conserving the threatened plants concerned. As per recent statistical reports, about 17,000 species of medicinal and aromatic plants are well documented and about 50,000 plant species are used globally. About 3,000 species are traded internationally and about 900 species are commercially cultivated worldwide. According to WWF (World Wide Fund for Nature), about 30 – 45% of medicinal plant species may be threatened with extinction in the wild, which means around 4000 to 10,000 plants are may be at risk. Various national and international agencies have formulated various policies and strategies for the conservation of medicinal plants.

Ethnomedicinal uses of Gidhya sag:

There are more than 6000 plant species are exist with their medicinal values but do not have scientific evidence, which is traditionally passed on orally through generation, out of which Gidhya sag is one of species. Those plants are required to conserve for future uses and to know their therapeutic activities through scientific research. In India the next 5^{th} person suffers from diabetes, in the present study mainly focuses on the conservation of medicinal plant locally named as (Gidhya sag) *Hymenodictyon obovatum* Wall mainly have medicinal property of antidiabetic medicine tribes uses bark of Gidhya Sag as water purifier.

It is a process of protecting endangered plants from threatened habitat and placing them in a new location which is in the wild area or within them of humans i.e., by cultivating and maintaining plants in biotic gardens, parks, other suitable sites.

National status: -

Nationally, *Hymenodictyon obovatum* Wall (Gidhya sag) plant has been explored by Vithal N Kadam (2003) Ethnobotanical survey of Umerkhed area and pharmacogenetic studies on some selected members. A PhD submitted to Amaravti university, Amaravati

Gokak *et al.*, (2012) as antimicrobial activity from Kumta and Dharwad, Karnataka, India. Growth promotion of Gidhya sag in forest has not been reported any authors.

International status:-

Internationally, no such work has been reported yet.

Significance of study: -

The present study will be helpful in isolation of PGPRB producing bacteria which will be effective for seed germination of *Hymenodictyon obovatum* Wall (Gidhya sag). The *Hymenodictyon obovatum* Wall (Gidhya sag) is a rare species which have medicinal value so conservation of this plant species is important which plays important role which affects on tribals cultural and their medicine practice.

6. Proposed Work Plan:

I six-months	Isolation of PGPRB (PSB and KSB) from rhizospheric niches.
II-six-months	Identification of effective PGPRB isolates. Deciphering of mechanism of PGPRB isolates for growth promotion.
III-six-months	Pot assay for growth promotion of Gidhya sag by using PGPR's isolates.
IV-six-months	Isolation of Phytopathogen from diseased Gidhya sag plant. Screening of PGPRB isolates against phytopathogens for biocontrol ability.
V- Six-months	Pot assay for biocontrol ability of PGPRB isolates against Gidhya sag phytopathogen.

7. Reference

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